Gene editing as a tool for developing cell-based models of a lysosomal storage disorder: preliminary results

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Introduction

Fabry Disease (FD), which has a prevalence between 1/3000 and 1/22,000, is one of the most common Lysosomal Storage Disorders (LSDs) (1). This disease is characterized by mutations in the α-galactosidase A (*GLA*) gene that will affect the structure and function of the α-Galactosidase A (α-GAL A) enzyme (2). Therefore, non-degraded globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3), accumulate in the lysosomes, leading to this multisystemic disease. Nowadays, the CRISPR/Cas 9 system gene editing tool is widely used in laboratories. In this technique, a single-guide RNA (sgRNA), which targets the local of interest in the genome, is added to the Cas9 enzyme that performs the double-strand break (DSB) in that locus (3). This enables gene editing through knockout (KO), knock-in (KI), insertions, and deletions.

Aims

In this work, we aimed to establish an **FD disease model using CRISPR/Cas9** system by **knocking out (KO) the HDFa iPSC line**.

We also aimed **to correct (KI) our nonsense mutation (p.W287X) in iPSCs derived from a patient with FD**.

The cell line used was produced in our laboratory, and registered in the Human pluripotent stem cell registry as INSAi002-A (4).

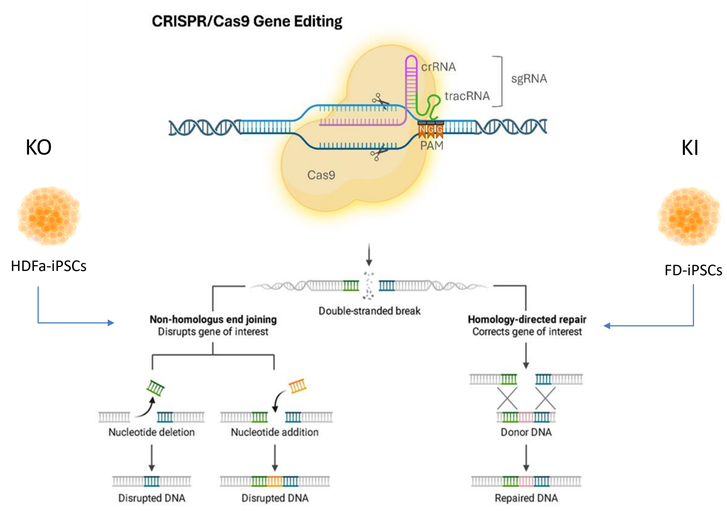

Figure 1: **Aims diagram**. *Created with BioRender.com*

Methods

sgRNAs for the HDFa-iPSCs KO and for the FD-iPSCs KI, were selected using the web tools Benchling (benchling.com), and ChopChop (chopchop.cbu.uib.no/). Cas9 Nuclease V3 (IDT™).

KO sgRNA sequence: 5′ -GCTAGCTGGCGAATCCCATG-3′

KI sgRNA sequence: 5′ -GAGCAGCCATGATAGCCTAG-3;

HDR donor: 5′ -GCCTCAGCTGGAATCAGCAAGTAACTCAGATGGCACTCTgGGCTATCATGGCTGCTCCTTTATTCATGTCT AATGACCTCGGCCTCAGCTGGAATCAGCAAGTAACTCAGATGGCCCTCTGGGCTATCATGGCTGCTCCTTTATTCATGTCTAAT GACCTC-3

The ribonucleoproteins (RNPs) – (sgRNAs + Cas9) - were transfected into the cells using the Neon™ Electroporation System (Invitrogen™) (Figure 2). The edits were analyzed using the Tracking of Indels by Decomposition (TIDE) tool for KO, and Tracking of Insertion, DEletions and Recombination events (TIDER) tool for KI.

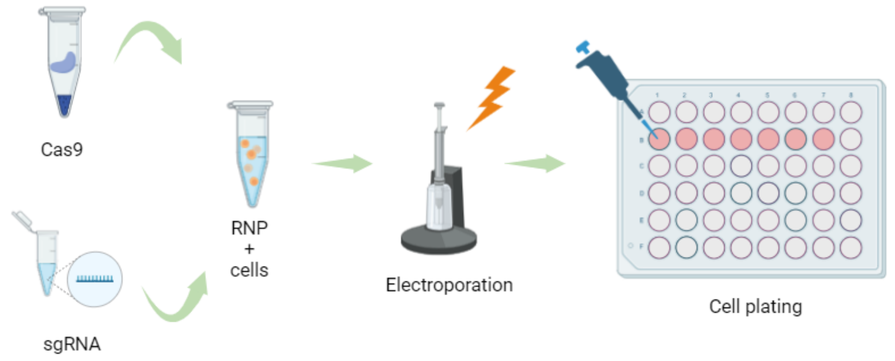

Figure 2: **Transfection workflow**. The RNPs are placed with the cells and after electroporation (1200V, 30 ms, 1 pulse) the cells are plated. *Created with BioRender.com*

Results

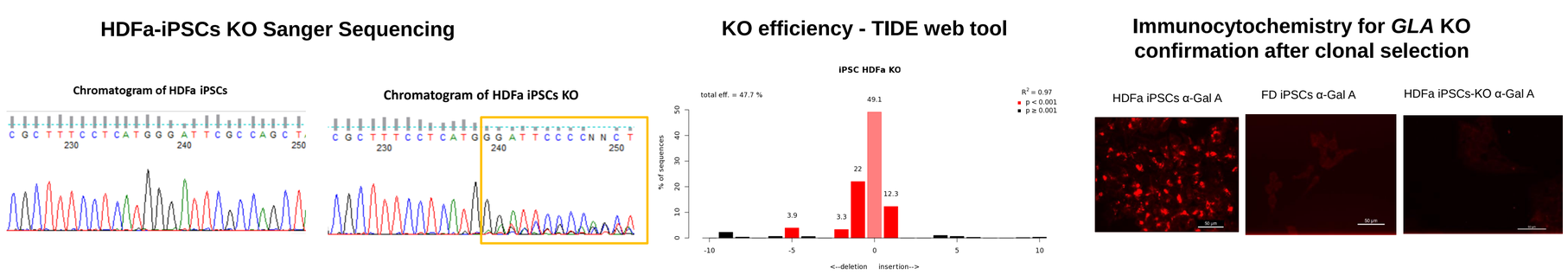

Figure 3: KO chromatogram, TIDE and immunocytochemistry analysis. The total editing efficiency at the desired locus was 49,1%. The α-Gal A protein was not detected in the immunocytochemistry.

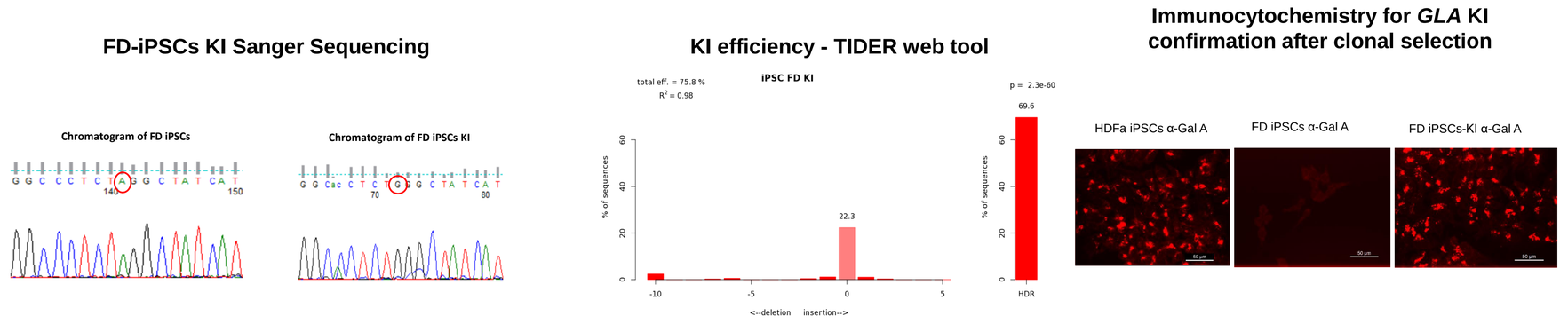

Figure 4: KI chromatogram, TIDER and immunocytochemistry analysis. The total editing efficiency was 75.8% being 69.6% of editing at the desired locus. The α-Gal A protein was not detected in the immunocytochemistry experiments.

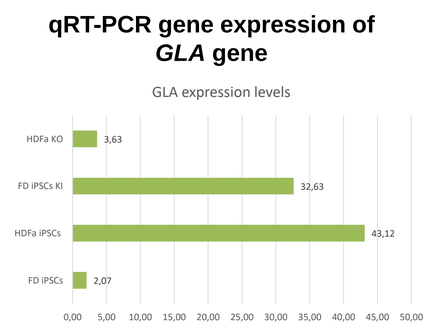

Figure 5: Western Blot for α-Gal A and qRT-PCR for *GLA* gene expression in all cell lines. The KI line present expression values similar to the control HDFa and the KO cell line present similar values to the patient's cells

Discussion and Conclusions

- We successfully generated an HDFa-iPS KO cell line for the *GLA* gene, that recapitulates the disease phenotype.
- In addition, we were able to correct the p.W287X mutation present in our FD-iPSCs, and rescue the normal physiological phenotype.
- To fully evaluate the molecular and cellular physiological changes, further studies are required.
- The development of innovative cell models, especially for rare diseases like LSDs, is beneficial for studying the pathophysiology of the disease.

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